(FILE 'HOME' ENTERED AT 18:09:08 ON 06 SEP 2002)

        FILE 'BIOSIS, MEDLINE, INPADOC, CAPLUS' ENTERED AT 18:09:22 ON 06 SEP 2002
L1   3 BONE CELL SPHEROID
L2   49 BONE AND SPHEROID
L3   46 L2 NOT L1
L4   32 DUPLICATE REMOVE L3 (14 DUPLICATES REMOVED)
L5   83937 CULTUR? AND (BONE OR OSTEO?)
L6   3521 L5 AND (TRANSFORMING GROWTH FACTOR#)
L7   238 L6 AND (SERUM FREE)
L8   6 L7 AND (BONE PRECURSOR CELL#)
L9   55 L7 AND OSTEOBLAST#
L10   30 DUPLICATE REMOVE L9 (25 DUPLICATES REMOVED)
L11   3135 (SERUM FREE MEDI##) AND ITS
L12   3135 (SERUM FREE MEDI##) AND "ITS+"
L13   124 (SERUM FREE MEDI##)(5A)"ITS+"
L14   60 DUPLICATE REMOVE L13 (64 DUPLICATES REMOVED)

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AN   1995:349833   CAPLUS
DN   122:123874
TI   Regulation of human **bone** marrow-derived **osteoprogenitor**
        cells by **osteogenic** growth factors
AU   Long, Michael W.; Robinson, J. A.; Ashcraft, E. A.; Mann, Kenneth G.
CS   Dep. Pediatr., Univ. Michigan, Ann Arbor, MI, 48109, USA
SO   Journal of Clinical Investigation (1995), 95(2), 881-7
        CODEN: JCINAO; ISSN: 0021-9738
PB   Rockefeller University Press
DT   Journal
LA   English
AB   Human **bone** marrow contains a distinct cell population that
        expresses **bone** proteins and responds to **transforming**
        growth factor .beta.1 (TGF-.beta.), but not to
        hematopoietic growth factors (Long, M. W. et al, 1990). The authors now
        report the isolation, characterization, and growth factor responsiveness
        of these precursors to human **osteoblasts** and the identification
        of a human **osteoprogenitor** cell. Immunol. sepn. of human
        bone marrow nonadherent low-d. (NALD) cells results in a marked
        enrichment of cells that express **osteocalcin**,
        osteonectin, and **bone** alk. phosphatase. Flow cytometric
        anal. show that distinct cell subpopulations exist among these isolated
        cells. The majority of the **bone** antigen-pos. cells are approx.
        the size of a lymphocyte, whereas other, less frequent antibody-sepd.
        subpopulations consist of **osteoblast**-like cells and
        osteoprogenitor cells. In **serum-free**
        cultures, TGF-.beta. stimulates the small, antigen-pos. cells to
        become **osteoblast**-like, as these cells both increase in size,
        and express increased levels of **osteocalcin** and alk.
        phosphatase. Antibody-sepd. cells also contain a sep. population of
        clonal progenitor cells that form colonies of **osteoblast**-like
        cells when **cultured** in **serum-free**,
        semi-solid media. Two types of human **osteoprogenitor** cells are
        obsd.: a colony-forming cell (CFC) that generates several hundred
        bone antigen-pos. cells, and more mature cluster-forming cell that
        has a lesser proliferative potential and thus generates clusters of 20-50
        antigen-pos. cells. **Osteopoietic** colony-forming cells and
        cluster-forming cells have an obligate but differential requirement for
        osteogenic growth factors. The CFCs respond to TGF-.beta., basic
        fibroblast growth factor (bFGF), **bone** morphogenic protein-2
        (BMP-2), and 1,25-dihydroxyvitamin D3 (1,25-OH D3). In contrast to the
        colony-forming cells, cluster-forming cells are regulated predominantly by
        1,25-OH D3 and TGF-.beta., but fail to respond to bFGF. The authors
        conclude that human **bone** marrow contains a nonhematogenous,
        heterogeneous population of **bone precursor**
        cells among which exists a population of proliferating
        osteoprogenitor cells. Further characterization of these
        bone precursor cell populations should yield
        important information on their role in **osteogenesis** in both
        health and disease.

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L1   ANSWER 3 OF 3   CAPLUS   COPYRIGHT 2002 ACS
AN   2000:628798   CAPLUS
DN   133:279465
TI   Three-dimensional cellular development is essential for ex vivo formation
      of human bone
AU   Kale, Sujata; Biermann, Sybil; Edwards, Claire; Tarnowski, Catherine;
      Morris, Michael; Long, Michael William
CS   Department of Pediatrics, Surgery, Chemistry, and Comprehensive Cancer
      Center, University of Michigan, Ann Arbor, MI, 48109, USA
SO   Nature Biotechnology (2000), 18(9), 954-958
      CODEN: NABIF9; ISSN: 1087-0156
PB   Nature America Inc.
DT   Journal
LA   English
AB   Tissue engineering of human bone is a complex process, as the functional
      development of bone cells requires that regulatory signals be temporally
      and spatially ordered. The role of three-dimensional cellular
      interactions is well understood in embryonic osteogenesis, but in vitro
      correlates are lacking. Here we report that in vitro serum-free
      transforming growth factor (TGF)-.beta.1 stimulation of osteogenic cells
      immediately after passage results in the formation of three-dimensional
      cellular condensations (bone cell spheroids) within 24 to 48 h. In turn,
      bone cell spheroid formation results in the
      up-regulation of several bone-related proteins (e.g., alk. phosphatase,
      type 1 collagen, osteonectin) during days 3-7, and the concomitant
      formation of micro-cryst. bone. This system of ex vivo bone formation
      should provide important information on the physiol., biol. and mol. basis
      of osteogenesis.
RE.CNT 24   THERE ARE 24 CITED REFERENCES AVAILABLE FOR THIS RECORD
              ALL CITATIONS AVAILABLE IN THE RE FORMAT

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AN 1978:194198 BIOSIS
DN BA66:6695
TI **BONE** CELLS A **SERUM-FREE** MEDIUM SUPPORTS
    PROLIFERATION IN PRIMARY **CULTURE**.
AU BURKS J K; PECK W A
CS DEP. MED., JEW. HOSP. ST. LOUIS, WASH. UNIV. SCH. MED., ST. LOUIS, MO.
    63110, USA.
SO SCIENCE (WASH D C), (1978) 199 (4328), 542-544.
    CODEN: SCIEAS. ISSN: 0036-8075.
FS BA; OLD
LA English
AB Bone cells isolated from embryonic rat calvaria increase in number
    2-3-fold when cultured at high, but not at low, population densities in a
    serum-free medium that contains albumin. Cultured cells respond to
    parathyroid hormone and exhibit a marked rise in alkaline phosphatase
    activity during proliferation, which suggests the progressive
    differentiation or preferential growth of osteoblast-like cells.

Bone Cells: A Serum-Free Medium Supports Proliferation in Primary Culture

Abstract. *Bone cells isolated from embryonic rat calvaria increase in number two-to threefold when cultured at high, but not at low, population densities in a serum-free medium that contains albumin. Cultured cells respond to parathyroid hormone and exhibit a marked rise in alkaline phosphatase activity during proliferation, which suggests t.. progressive differentiation or preferential growth of osteoblast-like cells.*

Studies in which isolated bone cells in primary culture are used have advanced our understanding of the actions of major bone-seeking hormones (*1*). Cultured cells are sensitive to near physiological concentrations of several hormones and retain the capacity to perform differentiated functions (*2, 3*). A strict requirement for serum to support the growth of bone cells in vitro, however, has impeded further clarification of the regulation of cell proliferation and differentiation, since serum contains defined and undefined growth-modifying substances (*4*). Attempts to grow nonskeletal cells in the absence of serum have met with but limited success. Additions of specific nutrients, hormones, or polypeptides reduce, but do not eliminate, serum requirements for growth of certain cell types in primary culture (*5*). Although several established cell lines have been found to grow in serum-free medium, they are less suitable than primary cultures for studies of differentiated functions (*5*). We report the development of a serum-free medium that supports the proliferation of isolated bone cells in primary culture.

Bone cells were isolated from the frontal and parietal bones of rat fetuses 19 to 21 days old, as previously described (*1*). Cartilage and periosteal connective tissue were removed by sharp dissection prior to enzymatic digestion with crude collagenase (*1*). Cultures were seeded at an initial density of 10^6 cells (10^5 cell/cm^2) per Falcon petri dish (diameter, 35 mm) and were maintained in a humid atmosphere of 2 percent CO_2 and 98 percent air. Cells were grown in the Fitton-Jackson BGJ_b medium (*6*), previously used to study bone cell metabolism (*7*), and modified so that its ionic composition resembled that of the bone fluid as described by Neuman and Ramp (*8*) (Table 1). The sodium and potassium concentrations were 125 mM and 25 mM, respectively. This medium (2.0 ml) was replaced three times a week. Cell number (after trypsinization), alkaline phosphatase activity, and DNA content were estimated on days 1, 5, 9, and 12 of culture (Fig. 1). Replicate cell cultures were exposed to [^{3}H]thymidine (1.0 μc per 2.0 ml of culture medium) (*9*) for 1 hour, and the radioactivity in the acid-insoluble fraction (*10*) was estimated

with a Packard Tri-Carb liquid-scintillation spectr ph tometer.

Cells began t attach to the plastic surface within 5 minutes f seeding. Although 35 percent of the inoculated cells remained firmly attached after the first 24 hours, failure to attach was not related to cell death, since 75 percent of the unattached cells were viable as judged by trypan blue exclusion. In view of the well-known limitations of dye exclusion methods in assessing cell integrity, the unattached cells may have been damaged sufficiently to prevent attachment. Alternatively, the attached cells may represent a different type of cell than the remainder of the aggregate cell harvest. Microscopic inspection of the culture plates suggested cell proliferation by day 5. This impression was confirmed by the presence of statistically significant increases in the cell number and total DNA that continued through day 9 (Fig. 1). The incorporation of [^{3}H]thymidine rose progressively to a maximum of 42,160 ± 2,232 disintegrations per minute (dpm) per culture (mean ± standard error of the mean) on day 7, then gradually fell to 17,237 ± 1,281 dpm per culture on day 12, by which time the cultures were nearly confluent. The conditions used in these experiments consistently supported bone cell proliferation. Figure 1 presents data accumulated from six separate experiments. Bone cells cultured at lower initial population densities (5×10^4 cells per square centimeter) failed to proliferate. Instead, there was gradual cell attrition over a 12-day period.
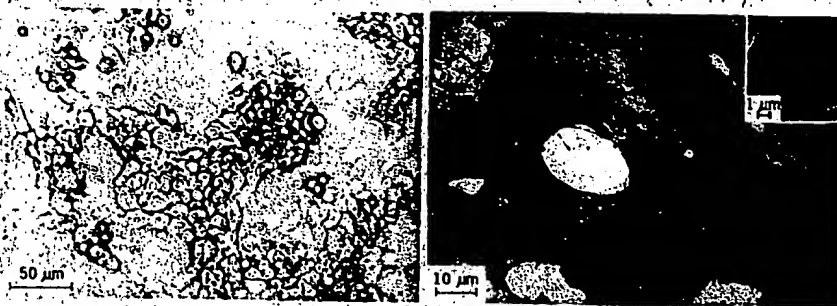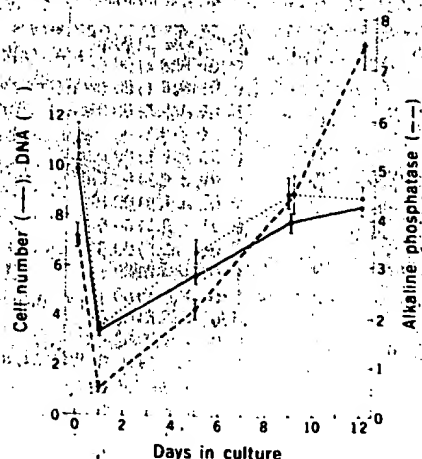
Three lines of evidence suggest the

Fig. 1 (left). Growth of isolated subperiosteal bone cells seeded at an initial population density of 10^5 cell/cm^2. Each point represents the mean and standard error of data pooled from six separate experiments. Day 0 represents the initial inoculation; all other points represent firmly attached cells removed from the plates after trypsinization. Solid line is cell number $\times$ 10^5 per culture; dashed line, alkaline phosphatase activity [(micromoles of *p*-nitrophenol per hour) $\times$ 10^3]; dotted line, DNA in micrograms per culture. Arrows represent the days on which the medium was replaced. Following enzymatic digestion of the calvaria, the dispersed cells were rinsed four times with isolation medium, allowed to stand at room temperature for 5 minutes in isotonic ammonium chloride to lyse red blood cells (*22*), and then washed and suspended in isolation medium for hemocytometer counting. Alkaline phosphatase activity was measured by the method of Koyama and Ono (*23*) at a pH of 10.3. DNA was determined by the indole method of Hubbard *et al.* (*24*), in which hydrolysis for 20 minutes at 90°C and a volume of 1.0 ml was used, or by the diphenylamine method of Leyra and Kelley (*25*). Both methods gave identical results when corrected for recovery of internal standards. Fig. 2 (right). Morphological characteristics of bone cells cultured for 12 days in bone fluid medium. (a) Phase-contrast photomicrograph demonstrating dense aggregates of cells in a near confluent monolayer. (b) Cytoplasmic and nuclear alkaline phosphatase activity. Cultures were fixed at 4°C (30 seconds) with 10 percent formalin in methanol and stained by the method of Ackerman (*11*). Inset represents the region where cells form dense aggregates. Cells devoid of alkaline phosphatase activity are not visible.

preferential growth r differentiation (r both) of oste blast-like cells in this culture system. (i) A marked rise in alkaline ph sphatase activity per cell accompanied cell growth (Fig. 1). (ii) The number of cells that c ntained prominent cyt plasmic alkaline phosphatase activity increased from less than 1 percent on day 1 to more than 25 percent on day 12 of culture (Fig. 2). (iii) Cells cultured for 9 to 12 days responded to parathyroid hormone [synthetic bovine parathyroid hormone, amino acids 1-34 (11, 12), 100 ng/ml for 5.0 minutes] with a tenfold increase in 3',5'-adenosine monophosphate (cyclic AMP) when treated as described (2) (data not shown). There is every reason to believe that osteoblasts contain copious amounts of cytoplasmic alkaline phosphatase (13). Luben et al. (14) have provisionally characterized as osteoblasts those bone cells that are rich in alkaline phosphatase and respond to parathyroid hormone with large increases in cyclic AMP. The observed increase in alkaline phosphatase activity might reflect recovery from damage to surface enzyme caused by exposure of the bone cell to collagenase during isolation, rather than de novo differentiation. In favor of the latter interpretation, however, is the finding of Miedema (15) that the loss of alkaline phosphatase activity which accompanied collagenase treatment of an established cell line was not reversible. Definitive identification of the surviving cells as osteoblasts will require the demonstration that they are capable of producing bone.

Miedema (15) has demonstrated a similar density-dependent increase in alkaline phosphatase activity in cultured human HEp2 and WISH cells, as have Binderman et al. (7) in an isolated bone cell system. In each case, the incubation medium was supplemented with fetal calf serum. Yamane et al. (16) have initiated primary cultures of ascites tumor cells in a defined medium supplemented with 1 percent fat-containing serum albumin. In addition to providing essential fats, albumin may have served to trap toxic substances (17). Fat-poor albumin, such as that used in our system, was not found to support the growth of the ascites cells (16).

To determine whether the unusual ionic composition of the incubation medium used in our experiments (125 mM sodium, 25 mM potassium) influenced cell growth and apparent differentiation, we examined the effect of a medium containing m re physiol gic concentrati ns f sodium (145 mM) and p tassium (5 mM). Direct c mparison f these media revealed no differences in plating efficiency or in the rate of cell proliferation. Cells cultured in the standard medium, however, exhibited only 50 percent of the maximum alkaline phosphatase activity and were nly 50 percent as responsive t parathyroid hormone (see above) as those grown in the low sodium–high potassium medium (data not shown). No proliferation was observed in medium containing 155 mM sodium and 25 mM potassium (mean osm lality, 385 milliosmoles). Hence proliferation may be sensitive t osmolality, and apparent differentiation to the c ncentrations of sodium and potassium.

Isolated peri steal cells, removed fr m the superi r and inferior peri steal surfaces by sharp dissection prior t collagenase digestion (18), failed to survive in the serum-free medium even when

Table 1. Comparison of Fitton-Jackson BGJ$_b$ with bone fluid medium. Fitton-Jackson modified BGJ$_b$ medium (Gibco) has a mean osmolality of 390 milliosmoles. To prepare the bone fluid medium, the salts and organic components are dissolved in 6 to 7 dl of glass-distilled deionized water. One deciliter of commercially prepared BGJ$_b$ (Fitton-Jackson modified) amino acids and vitamins (10 ×) without ascorbic acid and glutamine is added (Gibco, quotation No. 8202) followed by 1000 mg of Pentex albumin (Miles Laboratories). The pH is adjusted to 7.45 at room temperature with 0.4M NaOH and the volume adjusted to 1 liter; NaHCO$_3$ is added prior to membrane sterilization. The final pH is 7.50 to 7.55, which is optimum for cell attachment and collagen biosynthesis (21). The mean osmolality of the bone fluid medium is 350 milliosmoles. The isolation medium is prepared in a similar manner, but has a final pH of 7.00.

Component	Concentration	
	Fitton-Jackson BGJ$_b$ (mg/liter) (mole/liter)	Bone fluid medium (mole/liter) (mg/liter)
CaCl$_2$·2H$_2$O		73.52 (5.0 × 10^{-4})
Calcium lactate	555.00 (1.8 × 10^{-3})	
MgSO$_4$·7H$_2$O	200.00 (8.1 × 10^{-4})	98.60 (4.0 × 10^{-4})
K$_2$HPO$_4$		261.28 (1.5 × 10^{-3})
KH$_2$PO$_4$	160.00 (1.2 × 10^{-3})	
NaH$_2$PO$_4$·H$_2$O	90.00 (6.5 × 10^{-4})	41.40 (3.0 × 10^{-4})
NaCl	5,300.00 (9.1 × 10^{-2})	5,844.00 (1.0 × 10^{-1})*
KCl	400.00 (5.4 × 10^{-3})	1,640.26 (2.2 × 10^{-2})
NaHCO$_3$	3,500.00 (4.2 × 10^{-2})	1,260.60 (1.5 × 10^{-2})
FeSO$_4$·7H$_2$O		0.83 (3.0 × 10^{-6})
Hepes†		4,766.00 (2.0 × 10^{-2})
D-Glucose	10,000.00 (5.5 × 10^{-2})	2,000.00 (1.1 × 10^{-2})
Sodium acetate (3H$_2$O)	50.00 (6.0 × 10^{-4})‡	54.43 (4.0 × 10^{-4})
α-Ketoglutaric acid		29.22 (2.0 × 10^{-4})
Phenol red	20.00 (5.6 × 10^{-5})	5.00 (1.4 × 10^{-5})
L-Alanine	250.00 (2.8 × 10^{-3})	250.00 (2.8 × 10^{-3})
L-Arginine	175.00 (1.0 × 10^{-3})	175.00 (1.0 × 10^{-3})
L-Aspartic acid	150.00 (1.1 × 10^{-3})	150.00 (1.1 × 10^{-3})
L-Cysteine HCl·H$_2$O	90.00 (5.1 × 10^{-4})	90.00 (5.1 × 10^{-4})
L-Glutamine	200.00 (1.4 × 10^{-3})	292.3 (2.0 × 10^{-3})
Glycine	800.00 (1.1 × 10^{-2})	800.00 (1.1 × 10^{-2})
L-Histidine	150.00 (9.6 × 10^{-4})	150.00 (9.6 × 10^{-4})
L-Isoleucine	30.00 (2.3 × 10^{-4})	30.00 (2.3 × 10^{-4})
L-Leucine	50.00 (3.8 × 10^{-4})	50.00 (3.8 × 10^{-4})
L-Lysine	240.00 (1.3 × 10^{-3})	240.00 (1.3 × 10^{-3})
L-Methionine	50.00 (3.4 × 10^{-4})	50.00 (3.4 × 10^{-4})
L-Phenylalanine	50.00 (3.0 × 10^{-4})	50.00 (3.0 × 10^{-4})
L-Proline	400.00 (3.5 × 10^{-3})	400.00 (3.5 × 10^{-3})
L-Serine	200.00 (1.9 × 10^{-3})	200.00 (1.9 × 10^{-3})
L-Threonine	75.00 (6.3 × 10^{-4})	75.00 (6.3 × 10^{-4})
L-Tryptophan	40.00 (2.0 × 10^{-4})	40.00 (2.0 × 10^{-4})
L-Tyrosine	40.00 (2.2 × 10^{-4})	40.00 (2.2 × 10^{-4})
DL-Valine	65.00 (5.6 × 10^{-4})	65.00 (5.6 × 10^{-4})
α-Tocopherol phosphate	1.00 (2.3 × 10^{-6})	1.00 (2.3 × 10^{-6})
Ascorbic acid	50.00 (2.8 × 10^{-4})	50.00 (2.8 × 10^{-4})
Biotin	0.20 (8.2 × 10^{-7})	0.20 (8.2 × 10^{-7})
Calcium pantothenate	0.20 (4.2 × 10^{-7})	0.20 (4.2 × 10^{-7})
Choline chloride	50.00 (3.6 × 10^{-4})	50.00 (3.6 × 10^{-4})
Folic acid	0.20 (4.5 × 10^{-7})	0.20 (4.5 × 10^{-7})
i-Inositol	0.20 (1.1 × 10^{-6})	0.20 (1.1 × 10^{-6})
Nicotinamide	20.00 (1.6 × 10^{-4})	20.00 (1.6 × 10^{-4})
p-Amino benzoic acid	2.00 (1.6 × 10^{-5})	2.00 (1.6 × 10^{-5})
Pyridoxal phosphate	0.20 (8.1 × 10^{-7})	0.20 (8.1 × 10^{-7})
Riboflavin	0.20 (5.3 × 10^{-7})	0.20 (5.3 × 10^{-7})
Thiamine-HCl	4.00 (1.2 × 10^{-5})	4.00 (1.2 × 10^{-5})
Vitamin B$_{12}$	0.04 (2.9 × 10^{-8})	0.04 (2.9 × 10^{-8})

*The amount of NaCl added depends upon the NaCl content of the commercially obtained amino acid–vitamin mixture. The final ion concentrations are similar to the bone fluid described by Neuman and Ramp: Na$^+$, 125 mM; K$^+$, 25 mM; Ca^{2+}, 0.5 mM; Mg^{2+}, 0.4 mM; Cl$^-$, 130 mM; and P$_i$, 1.8 mM (8). †N-2-Hydroxyethylpiperazine-N'-2-ethanesulfonic acid. ‡Anhydrous sodium acetate.

seeded at high initial population densities (10^5 cell/cm²). Seventeen percent f the cells attached, but only 5 percent remained by day 5. The alkaline phosphatase activity was low (28.3 ± 5.3 μmole per h ur per culture) in comparis n with that f cell cultures prepared from subperi steal bone. Fibroblasts btained by collagenase digestion of fetal rat skin died over the initial 2- to 3-day period in culture.

Growth and apparent differentiation at a high, but not at a low, initial population density may reflect the continuing action f tissue or serum growth factors, or b th, that adhered to the cells during preparation. Alternate explanations include (i) the elaboration by cultured cells of their own growth factors or essential nutrients that are absent from the medium (19) and (ii) inactivation by the larger cell mass of toxic substances in the medium (20). The latter possibility would explain not only the failure of cells to proliferate when cultured at low density but also the low plating efficiency. Although there was appreciable cell death during the first 24 hours in culture, stimulation of proliferation cannot be attributed easily to growth-promoting factors released into the incubation medium by the dying cells. The incubation medium was replaced completely at 24 hours (Fig. 1), whereas proliferation was evident long afterward. Moreover, fibroblasts and periosteal cells did not proliferate. despite greater initial cell attrition. The system described herein should permit clarification of the mechanism of density-dependent proliferation and an examination of the effects of growth factors on the proliferation and differentiation of bone cells in vitro in the absence of complex and ill-defined serum additives.

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26. We thank J. Weil and I. Dowling for their technical assistance. This work was supported by research grant AM-19855 from the National Institute of Arthritis, Metabolism, and Digestive Diseases.

31 May 1977; revised 19 August 1977

Cyclopropanecarboxylic Acid: Chain Elongation to ω-Cyclopropyl Fatty Acids by Mammals and Plants

Abstract. Rats dosed orally with [carboxyl-¹⁴C]cyclopropanecarboxylic acid (or its hexadecyl ester) retain radioactivity in tissue as novel triacylglycerols. The most abundant ¹⁴C-labeled metabolites were identified by gas-liquid chromatography-mass spectrometry as 13-cyclopropyltridecanoic and 15-cyclopropylpentadecanoic acids. Similar ω-cyclopropyl fatty acids are produced by beagle dogs and a lactating cow, as well as by apple and orange trees.

Certain esters of cyclopropanecarboxylic acid (CPCA) are selectively toxic to phytophagous Acarina (spider mites) without significant insecticidal activity (1, 2). We have studied the metabolic fate of the hexadecyl ester of CPCA (cycloprate), which is currently under commercial development as a miticide. We have identified unusual metabolites arising from the apparent entry of CPCA into pathways of fatty acid anabolism, with net additions of acetate to give a homologous series of ω-cyclopropyl fatty acids.

Duncombe and Aising (3) studied the metabolism of ¹⁴C-labeled CPCA in rat tissue in vitro and postulated the formation of unsaturated ω-cyclopropyl fatty acids, but were unable to identify these products. Linscott et al. (4, 5) have shown that plants can elongate the carbon chains of 2,4-dichlorophenoxyalkanoic [for example, -acetic (2,4-D) and -butyric] acids by insertion of pairs of methylene groups. The major metabolites of these acids were identified as resulting from addition of one to three acetate units (that is, two to six CH_2 groups), whereas we find addition of up to eight acetates to CPCA. We know of

Table 1. Quantitative abundance of ω-cyclopropyl fatty acids, as a percent of the administered dose of [¹⁴C]cycloprate, in portions of various organisms that were analyzed (6).

ω-Cyclopropyl fatty acids*	Rat carcass	Cow milk	Dog carcass	Apple fruit	Orange fruit
8(5cPr):0		0.2			
10(7cPr):0		0.3			
12(9cPr):0		0.3			
14(11cPr):0	1.0	0.7	0.4		
16(13cPr):0	9.9	1.6	6.5		
18(15cPr):0	2.5	0.2	2.7	4.5	7.6
18(15cPr):1				1.9	3.7
20(17cPr):1					0.3

*Structural abbreviations are explained in the legend to Fig. 1.

TI   LONG-TERM ORGAN CULTURE OF EMBRYONIC CHICK FEMORA A SYSTEM FOR
      INVESTIGATING BONE AND CARTILAGE FORMATION AT AN INTERMEDIATE LEVEL OF
      ORGANIZATION.

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AB   Bone organ culture is an experimental system in which skeletal cells
      remain within their extracellular matrix but are removed from systemic
      influences. Femurs from 14-day-old chick embryos, which contain bone and
      cartilage matrix in approximately equal proportions, were **cultured**
      for up to 9 days in a **serum-free** medium. Cell
      proliferation, differentiation into chondrocytes and osteoblasts,
      formation of **bone** and cartilage matrix, and in vitro
      mineralization as well as bone and cartilage resorption were assessed
      using histologic and analytic methods. Particular attention was paid to
      the differences between cartilage and bone growth and to interpreting
      analytic data in the light of histologic observations. The first 2 days of
      culture represented an "adaptation" period, characterized by the release
      of intracellular enzymes into the culture medium, probably as a
      consequence of cell breakdown. Days 3-9 in culture represented a period of
      "steady growth" during which skeletal cells continued to multiply in the
      absence of fetal serum and to secrete large amounts of bone and cartilage
      matrix. De novo mineralization could be induced by Ca-.beta.-
      glycerophosphate, but calcium deposits in tissues other than bone and
      cartilage were also induced. Resorption of bone or cartilage matrix was
      virtually absent. Bone organ culture facilitates the study of bone and
      cartilage formation at an intermediate level of organization and thereby
      provides the necessary link between in vivo studies and investigations at
      the cellular level.